

# Function of Soluble CD14 in Serum from Patients with Septic Shock

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Soluble CD14 (sCD14) mediates lipopolysaccharide (LPS) activation of epithelial cells *in vitro* and may thereby be harmful in sepsis. sCD14 function was analyzed in sera from 62 patients with septic shock and compared with data from appropriate controls. sCD14 function was measured as sCD14-dependent LPS-induced interleukin (IL)-8 release in the SW620 epithelial cell line. In these cells, IL-8 production correlated with LPS concentration and the amount of sCD14. The effect of natural or recombinant sCD14 was maximal at 100 ng/mL and blocked by anti-CD14 antibodies. Patient and control sera (0.5% final concentration) promoted induction of IL-8 by 100 ng/mL LPS in SW620 cells. In sepsis patients (highest serum sCD14), values were significantly higher than in the other groups. The LPS-induced IL-8 response was blocked by anti-CD14 and correlated with the serum CD14 level in sepsis patients. Thus, sCD14 could play a pathogenetic role in sepsis.

Lipopolysaccharide (LPS) plays a key role in gram-negative sepsis. It binds to membrane CD14 (mCD14) on myeloid cells and to soluble CD14 (sCD14) in serum [1, 2]. LPS bound to the glycosyl-phosphatidylinositol (GPI)-linked mCD14 promotes monocyte activation via tyrosine phosphorylation of several kinases [3–5]. The LPS-sCD14 complex activates endothelial and epithelial cells, which are devoid of mCD14, via an unknown receptor [6–8]. sCD14 also acts on myeloid cells; it was shown to compete with mCD14 for LPS binding and to reduce LPS-induced cytokine production in monocytes and macrophages [9]. We have recently shown that patients with gram-negative septic shock have increased levels of sCD14 that are associated with high mortality [10]. However, the pathogenetic role of sCD14 in sepsis is unknown.

mCD14 is a 54-kDa glycoprotein [11] that was initially known as a myeloid differentiation marker [12]. sCD14 is released in two isoforms (49 and 55 kDa) from monocytes, both lacking the GPI anchor [10, 13, 14]. The smaller form is derived from the membrane by proteolytic cleavage and therefore has a shortened C-terminus without a GPI anchor. The larger form is directly released from an intracellular source, without attachment of a GPI tail [13–16]. In serum of healthy volunteers, we found only the 49-kDa form. In contrast, in patients with

septic shock, we detected either form, provided that their CD14 level was <3.5 mg/mL. However, sera from patients with high sCD14 levels (>3.5 mg/mL) contained exclusively the larger 55-kDa form [10]. Myeloid cells from patients with paroxysmal nocturnal hemoglobinuria (PNH) lack mCD14, since they have an acquired deficiency in the synthesis of the GPI anchor [17]. Nevertheless, their serum contains normal amounts of sCD14 of the larger isoform, presumably from an intracellular source [10]. Serum of these patients can be used to study the function of the large isoform of sCD14.

We analyzed the function of sCD14 in patients with septic shock. In addition, we asked whether there was a functional difference between the two sCD14 isoforms. For this purpose, we studied the effect of serum sCD14 on LPS-induced interleukin (IL)-8 release in an epithelial cell model. Sera from patients with PNH, who display only the large isoform, and from healthy controls, who all display the small isoform, were compared with those from patients with sepsis. The epithelial cell model was chosen because measurements are more reproducible in a cell line than in primary cultures (e.g., of endothelial cells). Moreover, epithelial cells are also involved in inflammation during sepsis by secreting cytokines [18], and disturbed alveolar epithelial cell function participates in the pathogenesis of acute respiratory distress syndrome [19]. In addition, IL-8 serum levels are often elevated in sepsis [20], and the increased concentrations in these patients may originate from an *in vivo* effect of LPS and sCD14 on epithelial cells. Therefore, we analyzed whether CD14 in serum from sepsis patients could mediate LPS-induced IL-8 production in an epithelial cell line.

## Methods

**Patients.** The study population consisted of 69 patients (45 male, 26 female) with septic shock, who were part of a previously published study comparing the efficacy of J5-hyperimmune globulin ( $n = 30$ ) with standard IgG ( $n = 39$ ; Sandoglobulin; Sandoz, Basel, Switzerland) [21]. Fifty-three patients had gram-

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Informed consent was obtained from all patients participating in the study and from the patients with paroxysmal nocturnal hemoglobinuria. The study was approved by the Ethical Committee of University Hospital, Basel, in accordance with the guidelines of the Swiss Academy for Medical Science.

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negative sepsis and 16 had gram-positive or fungal sepsis or no bacteriologic documentation. The median age was 56 years (range, 7–78). In all patients, blood was sampled at study entry, at which time the median arterial blood pressure was 90/50 mm Hg and the median duration of shock was 10 h (range, 2–144). Sera were aliquoted and kept at  $-70^{\circ}\text{C}$  until use. In addition, 6 patients (2 men, 4 women) with PNH (ages 24–49 years; median, 32) and 11 healthy controls (7 men, 4 women; ages 29–73 years; median, 51) were studied.

**Reagents.** LPS of *Salmonella abortus equi* (smooth) and *Escherichia coli* Re 595 (rough) and lipid A were gifts of C. Galanos (Max Plank Institut, Freiburg, Germany). The following purified antibodies were used: MEM18 (IgG1; gift of V. Bazil, Institute of Molecular Genetics, Prague) [22], RoMo1 (IgG2a; IBL, Hamburg, Germany), and goat polyclonal anti-CD14 antiserum (gift of R. Ulevitch, Scripps Research Institute, La Jolla, CA). Mouse monoclonal antibodies 3C10 (IgG2b, ATCC TIB 228) and 63D3 (IgG1, ATCC HB44) directed against CD14 were purified by affinity chromatography from hybridoma culture supernatants. Affinity-purified IgG2b from an irrelevant monoclonal mouse antibody (ATCC TIB94 anti-IA<sup>b</sup>) served as control. Human serum was a pool from 5 healthy donors. It contained  $2.61\text{ }\mu\text{g/mL}$  sCD14; it was not heat-inactivated because sCD14 is destroyed by 30 min of incubation at  $56^{\circ}\text{C}$  (data not shown). The serum contained LPS-binding protein (LBP), as it allowed LPS-binding to monocytes sensitive to anti-LBP antiserum [23]; the LBP level was  $5.7\text{ }\mu\text{g/mL}$  (determined by P. Tobias, Scripps Research Institute). sCD14 was immunodepleted from serum by adsorption to 63D3-coupled CNBr-Sepharose. After centrifugation, the serum contained no detectable sCD14, as determined in a CD14 ELISA [10].

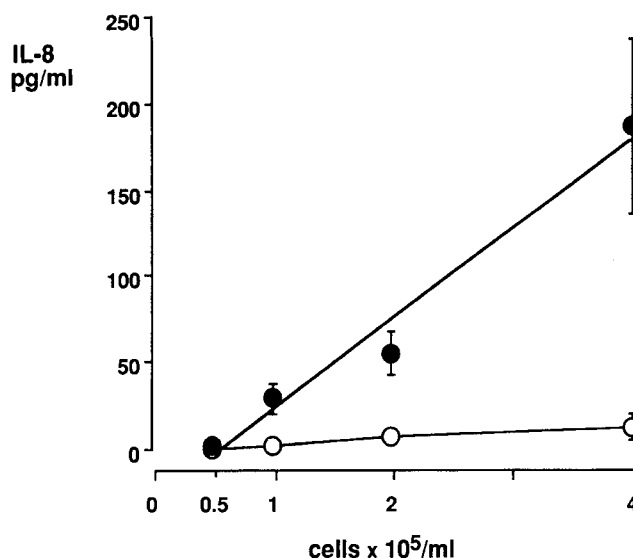
**Recombinant (r) sCD14 production and purification.** Oligonucleotide primers (sense, AGCACTCCAGAGCCTCTCC; antisense, GCAGCACCAGGGTTCCCGAC) were used to amplify a 1135-bp product. Plasmid that contained cDNA-encoding human CD14 served as a template (gift of R. Ulevitch). The PCR product was cloned into a eukaryotic expression vector (pRc, Invitrogen; Inotech, Dottikon, Switzerland). The expression construct was used to stably transfect CHO cells. Transfectants released  $10\text{ }\mu\text{g}$  of sCD14/ $10^6$  cells/24 h. rsCD14 was purified by immunoaffinity chromatography. For this purpose, anti-CD14 antibody 3C10, which does not compete with LPS for binding to sCD14 [24], was coupled to CNBr-activated Sepharose 4B (Pharmacia, Dübendorf, Switzerland). After treatment with  $1\text{ M}$  ethanolamine (pH 8.0) for 2 h at room temperature, the Sepharose resin was washed with TRIS buffer at increasing pH and molarity, treated with triethanolamine (pH 11) to eliminate nonspecifically bound antibody, and finally neutralized with PBS. The supernatant of transfected CHO cells, which had been cultured in RPMI medium with 2% fetal calf serum (FCS; GIBCO, Grand Island, NY) and  $100\text{ }\mu\text{g/mL}$  Geneticin (Boehringer, Mannheim, Germany), was adsorbed to 3C10-CNBr-Sepharose overnight at  $4^{\circ}\text{C}$ . After a wash with  $50\text{ mM}$  TRIS buffer (pH 8.0), elution with  $50\text{ mM}$  glycine buffer (pH 3.5) was started. The eluted fractions were tested for sCD14 by ELISA and Western blotting as described [10]. They did not contain any murine IgG. rsCD14 was aliquoted in PBS and stored at  $-70^{\circ}\text{C}$ .

**ELISA of sCD14.** Concentrations of sCD14 in sera from patients with sepsis were determined as described [10] by using the

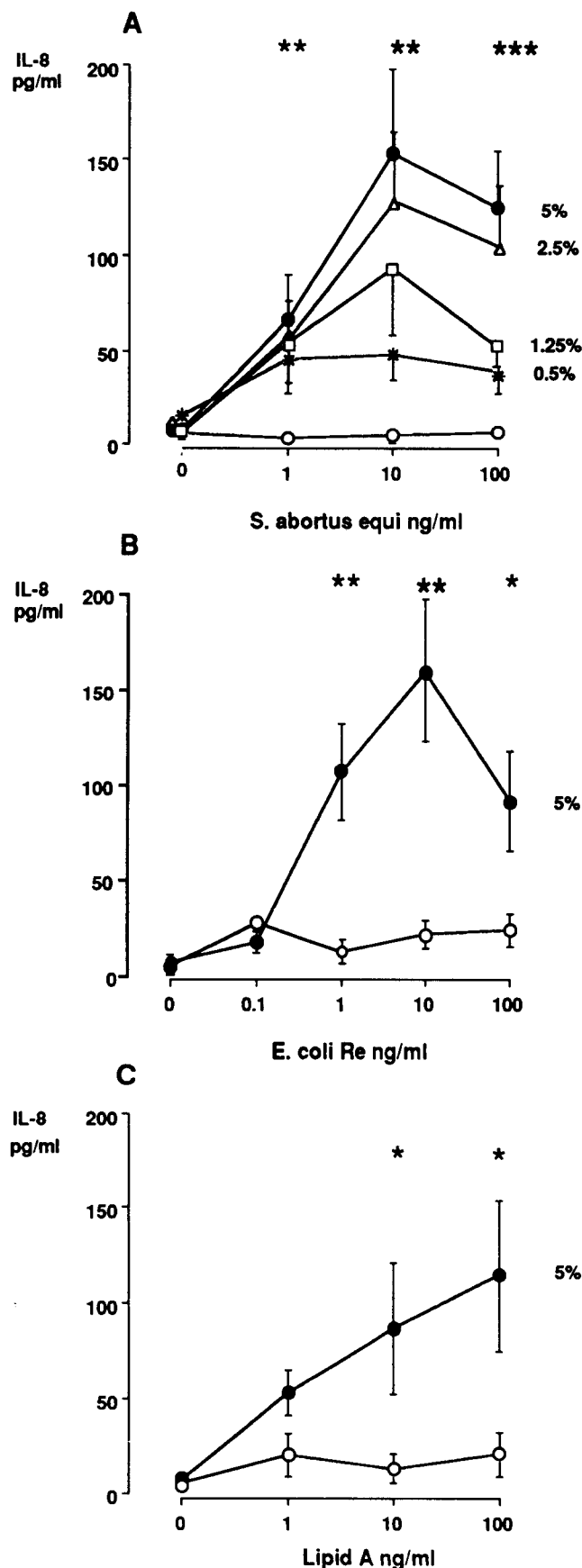
donated or commercially available anti-CD14 antibodies MEM18 and peroxidase-labeled RoMo1 with a calibrated serum as a standard. rsCD14 was analyzed by an ELISA that was developed in our laboratory with the use of anti-CD14 antibodies 63D3 ( $2\text{ }\mu\text{g/mL}$ ) and peroxidase-labeled 3C10 ( $500\text{ ng/mL}$ ).

**sCD14 functional assays.** SW620 cells (adenocarcinoma, ATCC CCL227) were maintained in RPMI 1640 with 10% heat-inactivated FCS supplemented with gentamicin. For the functional tests, cells were seeded into 96-well microtiter plates at  $4 \times 10^4$  cells/well and grown overnight. The cells were then washed twice in serum-free medium and stimulated the next 6 h with LPS in the presence of 2% human serum albumin or serum with or without the addition of antibodies.

**IL-8 ELISA.** Culture supernatants were harvested from the SW620 cells, centrifuged to eliminate cell debris, and kept at  $-20^{\circ}\text{C}$  for the determination of IL-8. Monoclonal anti-IL-8 antibodies ( $5\text{ }\mu\text{g/mL}$ ; provided by P. Stuetz, Sandoz) were coated onto 96-well polystyrene plates (Greiner, Frickenhausen, Germany) overnight at  $4^{\circ}\text{C}$ . Plates were washed three times with PBS–Tween 0.05%, and nonspecific binding was blocked for 2 h at  $37^{\circ}\text{C}$  with  $0.2\text{ M}$  TRIS-HCl buffer (pH 7.5) containing 1% bovine serum albumin and 0.05% Tween. Samples or standard ( $0.01$ – $10\text{ ng/mL}$  rIL-8; gift of M. Baggiolini, Theodor-Kocher-Institut, Bern, Switzerland) were incubated in  $0.1\text{ M}$  PBS with 0.5% FCS and 0.05% Tween for 2 h at room temperature. Plates were washed four times with PBS–Tween, then alkaline phosphatase-labeled monoclonal anti-IL-8 antibody ( $1.5\text{ }\mu\text{g/mL}$ , gift of P. Stuetz) was added and incubated for 2 h at  $37^{\circ}\text{C}$ . After a wash, the substrate *p*-nitrophenylphosphate (Sigma, St. Louis) was added for 1 h, and the enzymatic reaction was terminated by the addition of  $2\text{ M}$  NaOH. Absorbance was read at 405 nm in an ELISA reader (Mo-



**Figure 1.** Interleukin (IL)-8 release induced by  $100\text{ ng/mL}$  *Salmonella abortus equi* lipopolysaccharide (LPS) in 5% human pooled serum from SW620 cells at different cell concentrations ( $0.5$ ,  $1$ ,  $2$ , and  $4 \times 10^5$  cells/mL). ○, unstimulated cells; ●, LPS-stimulated cells. Means ( $\pm$ SE) of 3–5 experiments are shown. Regression analysis:  $r = .987$ ,  $P = .01$ .



lecular Devices, Palo Alto, CA). Human pooled serum did not contain detectable IL-8.

**Endotoxin measurement.** The endotoxin content in media, rsCD14, and antibodies was  $\leq 10$  pg/mL as determined by a limulus amoebocyte lysate assay kit (Chromogenix, Mölndal, Sweden).

**Statistics.** The results of the IL-8 assays in patients and controls and of assays with LPS in the presence and absence of serum were compared by analysis of variance and Scheffé's *F* test for post hoc evaluation of statistically significant differences. The relationship between two parameters was assessed by simple regression.

## Results

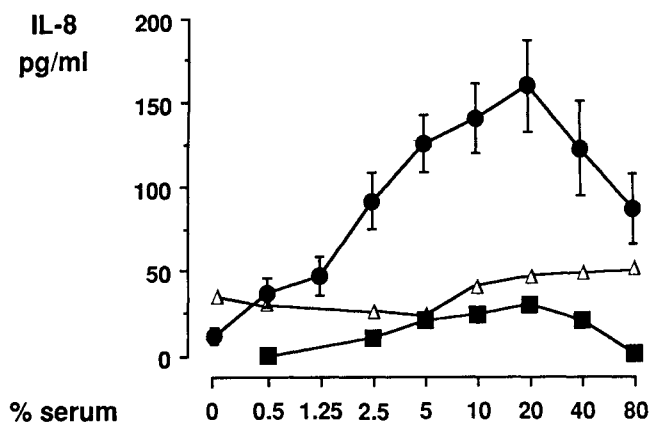
**Cell model used for the study of sCD14 function.** To measure sCD14 function in patient serum, the optimal assay conditions had to be defined. Therefore, we identified the relationships between cell number, serum concentration, LPS concentration, and IL-8 response in the SW620 cells.

Figure 1 shows a linear dose-response of IL-8 production on stimulation with 100 ng/mL LPS at cell concentrations between  $0.5$  and  $4 \times 10^5$  cells/mL ( $r = .987$ ,  $P = .01$ ). Without LPS, no IL-8 release was observed.

Since we wanted to investigate the effect of sCD14 on an LPS-dependent function in SW620 cells, we tested the dose response of IL-8 with different LPS preparations and concentrations. LPS from *S. a. equi* (smooth LPS, figure 2A) and from *E. coli* Re (rough LPS, figure 2B) induced a dose-dependent IL-8 release in the presence of 5% human pooled serum. Values peaked with 10 ng/mL smooth or rough LPS. LPS did not cause any IL-8 liberation in the absence of serum. The influence of the serum concentration is shown in figure 2A. At a suboptimal LPS concentration of 1 ng/mL, serum concentrations between 0.5% and 5% induced similar responses. In contrast, at 10 and 100 ng/mL LPS, the serum concentration was rate-limiting for IL-8 production. Stimulation with lipid A led to IL-8 production of lesser potency than did smooth or rough LPS (figure 2C).

Since LPS requires serum to induce epithelial cell activation [2] (figure 2), we determined the dose-response curve for serum with a high nonlimiting dose of LPS. As shown in figure 3, incubation of 100 ng/mL LPS in 0%–80% human pooled serum (corresponding to 0–2.1  $\mu$ g/mL sCD14) led to IL-8 liberation with a bell-shaped dose-response curve. The maximal ef-

**Figure 2.** Dose-response curve of lipopolysaccharide (LPS) on interleukin (IL)-8 release from SW620 cells without (○) or with 0.5%, 1.25%, 2.5%, or 5% serum: **A**, *Salmonella abortus equi* LPS. **B**, *Escherichia coli* Re LPS. **C**, Lipid A. Means ( $\pm$ SE) from 3 experiments are shown; values with 1–100 ng/mL *S. a. equi* and *E. coli* Re LPS as well as values with 10–100 ng/mL lipid A were significantly higher in presence than in absence of 5% serum. \*  $P < .05$ , \*\*  $P = .01$ , \*\*\*  $P < .001$ .



**Figure 3.** Effect of different concentrations of complete pooled serum (●), CD14-depleted serum (■), and pooled serum with 10 µg/mL anti-CD14 antibody 3C10 (△) on interleukin (IL)-8 release in SW620 cells stimulated with 100 ng/mL *Salmonella abortus equi* lipopolysaccharide. Means ( $\pm$ SE) of 3–8 experiments are shown.

fect was reached at 20% serum, corresponding to 520 ng/mL sCD14. At higher serum concentrations (i.e., 40% and 80%), the IL-8 response declined. LPS did not induce IL-8 release in CD14-depleted serum. At all serum concentrations, the LPS effect was blocked by anti-CD14 antibody 3C10.

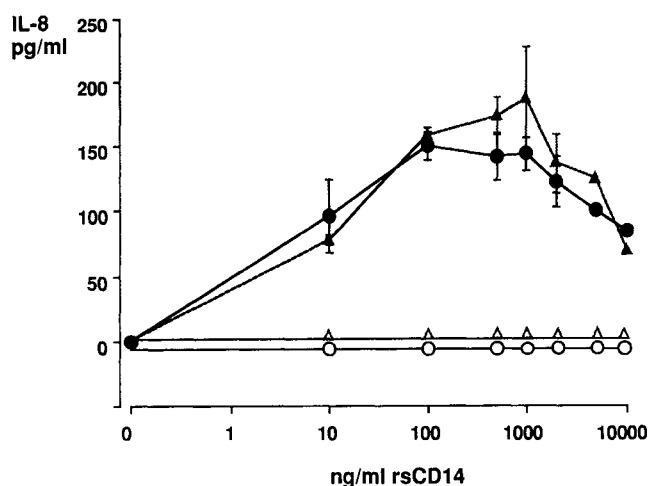
To further analyze the role of sCD14 in LPS-induced IL-8 production, blocking experiments were done with anti-CD14

antibodies. Figure 4 illustrates that LPS induced a similar amount of IL-8 with 5% human pooled serum (containing 130 ng/mL sCD14) and with 140 ng/mL rsCD14. In both, IL-8 production was blocked with anti-CD14 but not with control mouse IgG antibody (data not shown for rsCD14 + IgG). The 5% CD14-depleted serum did not promote the LPS effect on IL-8. Since the dose-response curve of serum was bell-shaped (figure 3), we also tested rsCD14 to see whether the same phenomenon occurred in the absence of serum. Figure 5 shows the dose-dependent IL-8 liberation with LPS and rsCD14. rsCD14 caused increasing IL-8 release at concentrations between 10 and 100 ng/mL. The effect plateaued from 100 to 1000 ng/mL and declined between 2 and 10 µg/mL rsCD14.

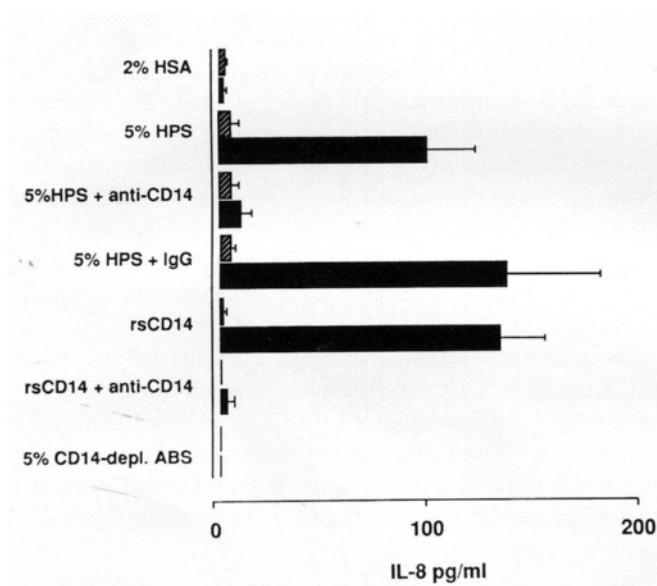
On the basis of the above-described results in SW620 cells, sCD14 dependence of IL-8 release in this model was demonstrated at low serum and corresponding sCD14 concentrations and at high LPS concentrations. These conditions were used to study patient sera.

*Effect of serum from patients with septic shock or PNH and from controls on LPS-induced IL-8 release.* We have previously noted elevated sCD14 levels in patients with gram-negative septic shock compared with healthy controls ( $3.61 \pm 0.26$  vs.  $2.48 \pm 0.81$  µg/mL) [10]. We extend this observation to 16 patients with septic shock due to non-gram-negative microorganisms (sCD14,  $4.01 \pm 0.61$  µg/mL). The 6 patients with PNH and the 11 healthy controls had mean sCD14 concentrations of  $3.08 \pm 0.71$  and  $2.56 \pm 0.14$  µg/mL, respectively. The values in patients with non-gram-negative sepsis were significantly ( $P < .05$ ) higher than in controls.

In the present study, the function of sCD14 was assessed in serum from 69 sepsis patients of both groups, from 6 patients with PNH, and from 11 healthy controls. There were elevated



**Figure 5.** Dose-response curves for effect of rsCD14 on interleukin (IL)-8 released with 100 ng/mL lipopolysaccharide (LPS). SW620 cells were treated with rsCD14 alone (○), with LPS plus rsCD14 (●), with LPS and rsCD14 plus IgG (▲), and with LPS and rsCD14 plus anti-CD14 (△).



**Figure 4.** Interleukin (IL)-8 production in unstimulated (▨) or lipopolysaccharide-stimulated (100 ng/mL *Salmonella abortus equi*, ■) SW620 cells. Cells were stimulated in medium with 5% complete human pooled serum (HPS), CD14-depleted serum (ABS), or 140 ng/mL rsCD14. Monoclonal anti-CD14 antibodies (10 µg/mL 3C10) or control mouse IgG2b hybridoma supernatant (10 µg/mL) was added to serum or rsCD14 before LPS stimulation. Means ( $\pm$ SE) of 4 experiments are shown. HSA, human serum albumin.

IL-8 levels in supernatants of unstimulated SW620 cells ( $73 \pm 24$  pg/mL) from 6 of 53 patients with gram-negative sepsis and 1 of 16 patients with non-gram-negative sepsis. This was due to a high serum IL-8 concentration ( $78 \pm 13$  ng/mL). After LPS stimulation, IL-8 did not further increase in these supernatants ( $74 \pm 15$  pg/mL). Therefore, these 7 patients were excluded from further analysis.

Figure 6 shows the IL-8 release in the epithelial cell model after stimulation with LPS and 0.5% serum from 47 patients with septic shock due to gram-negative organisms (figure 6A), 15 patients with sepsis due to non-gram-negative organisms (figure 6B), 6 PNH patients (figure 6C), and 11 healthy volunteers (figure 6D). Baseline IL-8 values did not differ statistically among the 4 groups. Patient and control sera allowed LPS to induce IL-8. Levels of LPS-induced IL-8 release were significantly higher in patients with non-gram-negative sepsis ( $81 \pm 9$  pg/mL) than in patients with gram-negative sepsis ( $43 \pm 4.0$  pg/mL,  $P < .001$ ), PNH patients ( $20 \pm 3.9$  pg/mL,  $P < .001$ ), or controls ( $34 \pm 3.5$  pg/mL,  $P < .001$ ). This difference was also observed in samples treated with LPS and the control antibody. Among patients with gram-negative sepsis, those with the 55-kDa isoform had a stronger LPS-induced IL-8 increase than those with the 49-kDa sCD14 ( $P = .02$ , data not shown). There was no significant difference in the IL-8 re-

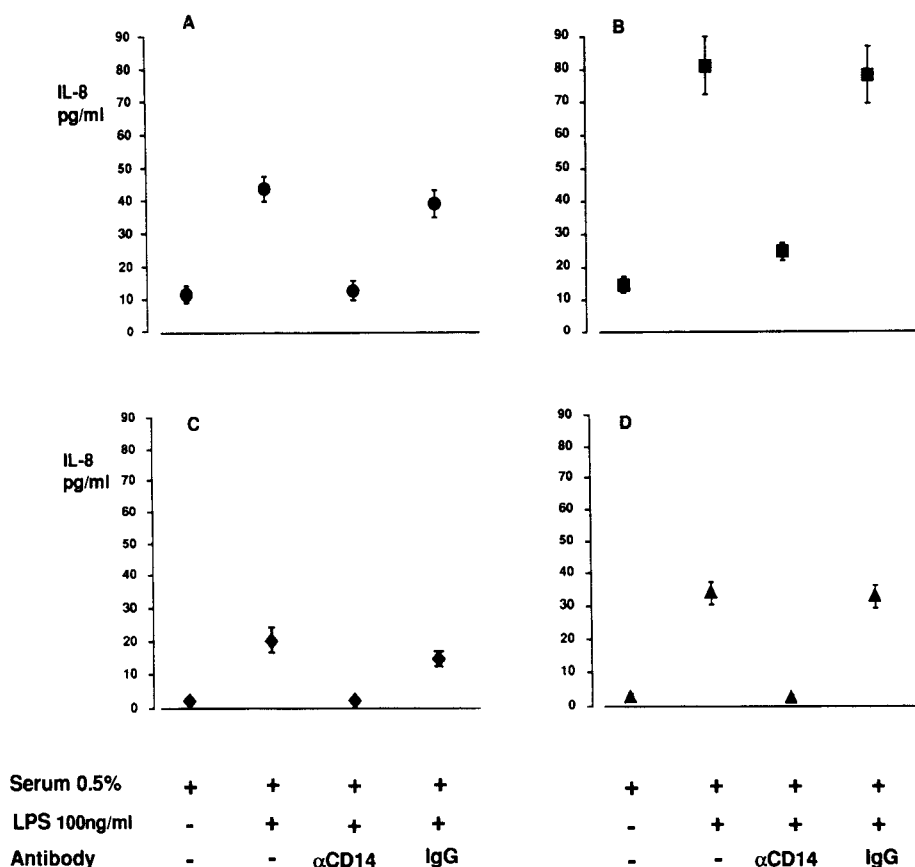
sponse between survivors and nonsurvivors. CD14 antibodies blocked LPS-induced IL-8 secretion in all samples, whereas control mouse IgG had no effect.

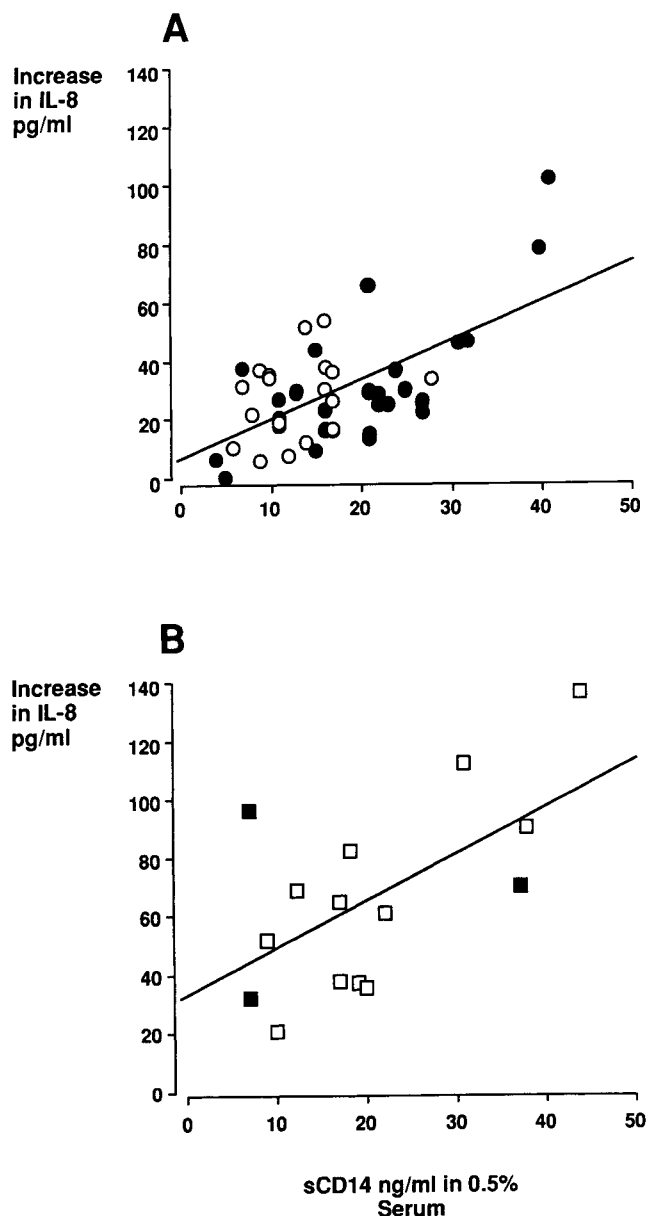
To confirm the dose-dependent effect of serum sCD14 in LPS-induced epithelial cell activation, the relationship between the IL-8 response and the sCD14 concentration in the assay (e.g., sCD14 in 0.5% serum) was established. In patients with gram-negative sepsis ( $n = 47$ ; figure 7A,  $P = .02$ ) or non-gram-negative sepsis ( $n = 15$ ; figure 6B,  $P = .01$ ), the increase in IL-8 with LPS stimulation correlated significantly with the sCD14 concentration. In gram-negative sepsis, only nonsurviving patients with the highest sCD14 concentration contributed to this relationship (nonsurvivors,  $r = .686$ ,  $P < .001$ ; survivors,  $r = .242$ , not significant).

## Discussion

The aim of the present study was to assess sCD14 function in sera of patients with septic shock. Sera from patients with PNH and from healthy volunteers were used as controls to measure the sCD14-dependent LPS effect in vitro. An epithelial cell line that responds to LPS with IL-8 release only in the presence of sCD14 was used as a model. sCD14 binds LPS and mediates its effects on cells devoid of membrane CD14

**Figure 6.** Effect of 0.5% serum on interleukin (IL)-8 release in unstimulated SW620 cells. Cells were stimulated with lipopolysaccharide (LPS) and without or with 10  $\mu$ g/mL monoclonal anti-CD14 antibodies or 10  $\mu$ g/mL control mouse IgG2B hybridoma supernatant. Serum from 47 gram-negative sepsis patients (A), 15 non-gram-negative sepsis patients (B), 6 paroxysmal nocturnal hemoglobinuria patients (C), and 11 healthy controls (D).





**Figure 7.** Relationship between serum CD14 concentrations and interleukin (IL)-8 response in SW620 cells. **A**, sCD14 concentration in 0.5% serum from 47 patients with gram-negative sepsis (○, survivors; ●, nonsurvivors;  $r = .686$ ,  $P < .001$ , line of best fit drawn for nonsurvivors only). **B**, 0.5% serum from 15 patients with non-gram-negative sepsis (□, survivors; ■, nonsurvivors;  $r = .632$ ,  $P < .01$ ).

[2, 6]. The dose-response curve for the LPS-sCD14-induced IL-8 release from epithelial cells has hitherto not been described. In particular, the potency of sCD14 at its normal serum concentration is unknown.

Therefore, we obtained dose-response curves with serum containing sCD14. We established that sCD14 activates epithelial cells with a bell-shaped dose-response curve. The maximal response occurred at 20% serum, corresponding to 500 ng/mL sCD14. At higher serum concentrations, LPS-induced IL-8 pro-

duction was weaker. This may have been due to a serum inhibitor, such as serum lipoproteins, which are known to neutralize LPS-sCD14 complexes [25]. However, a bell-shaped curve was also observed with rsCD14, with an optimal response at 100–1000 ng/mL and a weaker effect at 10  $\mu$ g/mL. Thus, rsCD14 was similar in potency to serum CD14. This finding, with LPS and CD14 in the absence of serum proteins, indicates that our experiments were done at an optimal ratio between LPS and CD14.

Serum contains LPS-binding protein (LBP), which enhances LPS affinity to myeloid, endothelial, or epithelial cells [26, 27]. We could exclude the role of LBP in our model, as LBP displays its activity only at low LPS concentrations [2, 27], and we used a high LPS concentration. This is in agreement with other observations of an LBP-independent sCD14-dependent adherence molecule expression by LPS in endothelial cells [27]. In addition to its agonist effect on epithelial cells, rsCD14 has been shown to neutralize LPS-induced tumor necrosis factor (TNF) release in human blood and chemiluminescence from monocytes at doses of 10–100  $\mu$ g/mL [9, 28]. In view of this dual role of sCD14 in vitro, it was interesting to test sCD14 function in sera from patients with septic shock ex vivo.

We first determined model conditions under which sCD14 and not LPS determined the IL-8 release. We found a dose dependence for sCD14 only at high LPS and low sCD14 concentrations (15–500 ng/mL) corresponding to 0.5%–20% serum. Since sepsis patients had normal or increased serum sCD14 levels with up to 5-fold higher values than controls, we used a low serum concentration to show an sCD14-dependent effect. Sera from patients and controls were effective in stimulating IL-8 release. This response was dependent on sCD14, because it was blocked by anti-CD14 but not by an irrelevant mouse antibody, and it was significantly related to the sCD14 concentration in serum.

Patients with non-gram-negative sepsis had the highest sCD14 serum levels and the strongest sCD14-dependent IL-8 response. This shows that the increase of serum sCD14 we observed in gram-negative sepsis was not specific for this disease and not induced by LPS [10]. It has recently been found that sCD14 not only binds to LPS but also to cell wall components from gram-positive microorganisms or mycobacteria [7, 29–31]. Therefore, probably not only LPS but also other bacterial components can cause an increase in serum sCD14. Alternatively, an intermediate product that is induced by gram-negative and other microorganisms induces the rise of serum CD14. Since we showed that IL-8 response correlated with serum CD14 concentration, sCD14 may represent the common mediator of epithelial [19] or endothelial cell effects [32] in sepsis due to different microorganisms. This hypothesis is sustained by earlier observations of elevated CD14 levels in serum or bronchoalveolar lavage fluid of infectious patients with poly-trauma, burns, or adult respiratory distress syndrome [33, 34]. The fact that the IL-8 response was stronger in gram-positive or fungal sepsis patients than in gram-negative sepsis patients

may indicate that in the former group, sCD14 activity was not neutralized by lipoproteins [25].

sCD14 from the patients was also tested to determine whether the predominance of one or the other sCD14 isoform (49 or 55 kDa) was associated with a different functional capacity. CD14 mediated the LPS effect irrespective of the predominant isoform. Although there was a higher response in the patients with the 55-kDa form than in those with the 49-kDa form, the difference was modest, and in all patients, the function was dependent on the sCD14 level. If the 55-kDa form differed in its functional capacity, sCD14 levels would not correlate with the IL-8 response in patients with the highest serum sCD14 level, who exclusively expressed the 55-kDa form.

Moreover, the difference would have been evident in the sera from PNH patients, in which we detected exclusively the 55-kDa isoform. Other authors, by using 12.5% polyacrylamide gels under reducing conditions and Western blot analysis of serum CD14, observed both isoforms in patients with PNH and in controls [14]. In our system, under nonreducing conditions in 7.5% polyacrylamide gels, we detected only the smaller form in healthy controls and the larger form in PNH patients and sepsis patients with high sCD14 levels. Thus, the predominant isoforms that we detected in each group did not appear to differ in their functional capacity. Since the two isoforms differ in their C terminal only, this result was expected. It has recently been shown that sCD14 from which two-thirds of the carboxy-terminus is deleted retains bioactivity [35].

Endothelial cells are activated not only by LPS but also, and more strongly, by the secondary mediators TNF and IL-1 [2]. Thus, sCD14 may serve purely as an initial trigger when LPS concentrations are very high. Alternatively, the direct LPS-sCD14 activation pathway may become important at a later stage of sepsis, when myeloid cells are adapted (i.e., when they no longer respond to LPS with a strong cytokine response). Moreover, it is possible that LPS-sCD14 complexes are activators of epithelial cells in the extravascular space, where myeloid cells are rare.

In conclusion, we have obtained evidence that sCD14 in serum from patients with septic shock activates epithelial cells in the presence of LPS. In this model we could not detect any LPS-neutralizing role of sCD14.

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